

Polymorphisms of Coagulation Factor XIII Subunit A and Risk of Nonfatal Hemorrhagic Stroke in Young White Women

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Background and Purpose—Although family studies have suggested a genetic influence on hemorrhagic stroke, the underlying genetic risk factors remain poorly defined. Coagulation factor XIII, which is involved in hemostasis, fibrinolysis, vascular remodeling, and tissue repair, represents a candidate gene for hemorrhagic stroke. We assessed the potential role of 3 factor XIII subunit A coding-sequence polymorphisms, along with a promoter polymorphism of plasminogen activator inhibitor-1 (PAI-1, which is also involved in fibrin stabilization and vascular remodeling), in young white women with hemorrhagic stroke.

Methods—Genotype analysis for factor XIII subunit A Val34Leu, Tyr204Phe, and Pro564Leu and for PAI-1 –675 4G/5G was performed in a population-based case-control study of 42 white women aged <45 years with nonfatal hemorrhagic stroke and 345 demographically similar control subjects.

Results—Compared with the respective homozygous wild-type genotypes, the Tyr204/Phe204 genotypes (age-adjusted odds ratio [OR] 2.9, 95% CI 1.1 to 7.5) and the Leu564/Leu564 genotype (OR 4.3, 95% CI 1.4 to 13.7) were each associated with an increased risk of nonfatal hemorrhagic stroke. The risk estimate associated with the Phe204 variant was highest in women with subarachnoid hemorrhage and in nonsmokers, whereas the risk estimate of the Leu564/Leu564 genotype was highest in women with intracerebral hemorrhage and in smokers. Women who carried either the Phe204 allele or the Leu564/Leu564 genotype in combination with the PAI-1 5G/5G genotype had a nearly 20-fold increased risk of hemorrhagic stroke (OR 18.9, 95% CI 3.8 to 95.1).

Conclusions—Our findings suggest that the Phe204 and Leu564 variants of coagulation factor XIII may be markers for genetic susceptibility to hemorrhagic stroke in women aged <45 years. (*Stroke*. 2001;32:2580-2587.)

Key Words: factor XIII ■ hemorrhagic stroke ■ plasminogen activator inhibitor-1

Hemorrhagic stroke occurs when an artery ruptures and causes blood leakage into the brain parenchyma or the subarachnoid space. Overall, it accounts for ≈20% of stroke victims and is the most common form of stroke among young adults. Data from family studies have suggested the importance of genetic influence in hemorrhagic stroke, particularly subarachnoid hemorrhage (SAH).¹⁻³ However, the specific genetic factors are poorly defined.⁴ Because intracranial aneurysms are associated with rare inherited connective tissue disorders, most previous candidate gene studies have focused on structural vessel wall proteins, such as collagen, or, in the case of intracerebral hemorrhage (ICH), cerebral amyloid deposition.^{4,5}

The events required for a hemorrhagic stroke to become clinically manifest include not only the formation of a weakened or abnormal vessel wall but also vessel rupture and hemorrhage. Inherited deficiency of coagulation factor XIII is

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a very rare autosomal-recessive bleeding disorder characterized by a particularly high rate of intracranial hemorrhage (up to 30% of cases).⁶ Coagulation factor XIII is a proenzyme that circulates as a heterotetramer composed of 2 catalytic subunits (factor XIII subunit A [factor XIIIa]) and 2 carrier subunits (factor XIII subunit B [factor XIIIb]). During the final stages of coagulation, thrombin cleavage of factor XIIIa results in formation of activated factor XIII, a transglutaminase that cross-links adjacent fibrin molecules to increase clot stability and resistance to fibrinolysis. Factor XIII also participates in extracellular matrix remodeling, cell adhesion and migration, and tissue repair.⁷ In a rat model of experimental cerebral aneurysm formation, exogenous administration of factor XIII abrogated the defective intimal proliferative response to arterial wall injury.⁸ Thus, deficiency of

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TABLE 1. Genotyping Assays for Factor XIII and PAI-1 Polymorphisms

| Polymorphism | Primers | PCR Product | Restriction Enzyme |
|-----------------------|--|------------------|--------------------|
| Factor XIII Val34Leu | CATGCCTTTTCTGTGTCTTC TACCTTGCAGGTTGACGCCCCGGGGCACTA | 192 bp (161, 31) | DdeI |
| Factor XIII Tyr204Phe | GGAAACAGTCTGGTTTGGTAA ACCCCGATGTCATTGAGGACG | 113 bp (91, 22) | RsaI |
| Factor XIII Pro564Leu | TCACCTTCTACACGGGGTCCG GACAGCGAGTCTCACAAGAA | 116 bp (95, 21) | BstUI |
| PAI-1 -675 4G/5G | CACAGAGAGAGTCTGGACACGTGA TGCAGCCAGCCACGTGATTGCTCTAG | 148 bp (110, 38) | BseRI |

Primer sequences are listed from 5' to 3', and the upper sequence represents the forward primer, the lower sequence, the reverse primer. The underlined bases were changed from the native sequence to introduce a restriction enzyme recognition sequence that would result in digestion of the PCR product to the fragments shown in parentheses for 1 (ie, Leu34, Tyr204, Leu564, or 4G) of the 2 alleles. Genomic DNA (50 ng) was amplified in a standard 20- μ L PCR reaction for 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 5 minutes. Aliquots of the PCR products were digested with the appropriate restriction enzyme for 1 hour according to the manufacturer's instructions (New England Biolabs).

coagulation factor XIII may contribute to the occurrence of hemorrhagic stroke through direct effects on vessel wall integrity or aneurysm formation in addition to bleeding tendency.

A common factor XIII gene nucleotide substitution (polymorphism) results in a Val34Leu amino acid substitution and was recently associated with increased risk of primary ICH in a predominantly elderly patient population.⁹ Two other common factor XIII polymorphisms, Tyr204Phe and Pro564Leu, have been associated with decreased plasma factor XIII levels,^{10–12} but their roles in hemorrhagic stroke have not been previously assessed. Therefore, we examined the association of the factor XIII Val34Leu, Tyr204Phe, and Pro564Leu polymorphisms with hemorrhagic stroke by using samples from a population-based case-control study of women aged <45 years. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of fibrinolysis and, like factor XIII, is involved in fibrin stabilization, vascular remodeling, and tissue repair.¹³ The PAI-1 -675 4G/5G promoter polymorphism has been associated with variation in plasma PAI-1 levels¹⁴ and with the risk of early-onset stroke¹⁵ or cerebrovascular mortality in women.¹⁶ Thus, we additionally assessed potential gene-gene interactions between the PAI-1 4G/5G polymorphism and the factor XIII polymorphisms.

Subjects and Methods

Study Subjects and Data Collection

The source population for the present study was women aged 18 to 44 years residing in 3 contiguous counties in western Washington state between 1991 and 1995 and represented ≈ 2.2 million women-years at risk.^{17,18} Eligible stroke patients free of prior cerebrovascular disease were initially identified by chart review of discharge diagnoses from all 34 hospitals and Emergency Medical Service incident reports in the study region. Stroke was defined as evidence of new focal neurological deficit(s) with no other apparent cause (ie, brain tumor, infection, subdural hematoma, seizure, or other neurological condition) lasting >24 hours. On the basis of reviews of hospital records, brain imaging studies, and lumbar puncture results, 141 of the 249 eligible stroke cases were classified as hemorrhagic by the study neurologist (W T L). Of the 141 hemorrhagic strokes, 91 were

further characterized as SAH on the basis of either (1) blood within the subarachnoid space in the presence or absence of a demonstrated aneurysm and no evidence of arteriovenous malformation or (2) an aneurysm with blood in other locations (eg, the parenchyma or ventricles) and no evidence of arteriovenous malformation. For the remaining 50 women, the strokes were classified as ICH. Six hundred eighty-four control women aged 18 to 44 years without history of cerebrovascular disease were identified from the same geographic area and were frequency-matched to the age distribution of the cases.

Of the 141 identified hemorrhagic stroke cases, 102 were alive and not disabled at the time of recruitment. Eighty (78.4%) of the 102 patients with nonfatal hemorrhagic stroke and 526 (76.9%) of the 684 eligible control subjects agreed to an in-person interview. Demographic characteristics and medical histories were ascertained during the interview, and blood samples for DNA isolation were collected from 54 hemorrhagic stroke cases (37 SAH and 17 ICH) and from 391 control subjects. Case blood samples were obtained at least 3 months after the stroke (mean 8 months). All study subjects gave informed consent according to a protocol approved by the University of Washington human subjects committee and participating local hospitals. Data collection for the present study did not include information regarding the use of ephedra compounds.

Factor XIII and PAI-1 Genotyping

Genomic DNA samples were prepared as described previously.¹⁸ Genotyping for the factor XIII Val34Leu polymorphism was performed by polymerase chain reaction (PCR) amplification of genomic DNA followed by restriction enzyme digestion (PCR-RFLP) with DdeI.¹⁹ Similar PCR-RFLP genotyping methods were developed for the Tyr204Phe and Pro564Leu factor XIII and PAI-1 4G/5G polymorphisms, as shown in Table 1. For all PCR amplifications, positive control DNA samples of known genotype as well as a negative control without template DNA were included. Ten percent of the study samples underwent repeat genotyping analysis, and the initial results were confirmed in all cases. As an additional validation measure, direct nucleotide sequencing of the PCR products was performed for selected homozygous or heterozygous individuals; in every case, the sequencing results agreed with the PCR-RFLP genotyping result.

Statistical Analysis

Factor XIII allele frequencies were calculated by gene counting. Genotype distributions and allele frequencies of each factor XIII polymorphism were compared among different groups by the χ^2 test.

To assess the relationship between the inheritance of allelic variants at one polymorphic locus and the allelic variants at a second polymorphic locus, pairwise linkage disequilibrium between factor XIII polymorphic loci was determined among controls by using a permutation procedure of the expectation-maximization algorithm²⁰ and expressed as D' , which ranges from 0 (complete linkage equilibrium) to 1 (complete linkage disequilibrium). D' is reported as positive when the minor allele at one polymorphic locus is associated with the minor allele at the other locus and is reported as negative when the minor allele at one locus is associated with the major allele at the other locus.

The association of factor XIII genotype with hemorrhagic stroke was examined by unconditional logistic regression adjusted for age and was expressed as odds ratios (ORs) and 95% CIs. Homozygosity for the more common allele (ie, Val34/Val34, Tyr204/Tyr204, or Pro564/Pro564) was used as the reference group in the regression models. In the case of the Val34Leu and Pro564Leu polymorphisms, 2 dummy variables representing the heterozygous and rare homozygous genotypes were modeled to provide separate estimates of association for each genotype. We also performed a multivariate logistic regression model that included all 3 factor XIII polymorphisms simultaneously to adjust for the potential confounding effects of the 2 remaining polymorphisms. Because the present study was exploratory in nature, in some instances we examined the combined risk estimate associated with the presence of heterozygosity for Phe204 or homozygosity for Leu564 on the basis of post hoc analysis of our data.

The extent to which associations with factor XIII genotypes were modified by other putative stroke risk factors was assessed through analyses stratified by these other risk factors. To test for the presence of interaction, multiplicative terms were introduced into the logistic regression models, and the P value was computed for the likelihood ratio test by comparing the model containing the interaction term with the model lacking the interaction term. Logistic regression models were performed by using Stata (version 6.0),²¹ and genetic data analysis was performed by using Arlequin (version 2.000).²² All statistical testing was 2-sided and performed at the $\alpha=0.05$ level.

Analysis of Factor XIII Alleles Among Healthy Black Subjects

Of the 54 hemorrhagic stroke cases and 391 control subjects with analyzable DNA, race was self-reported in 12 cases and 46 controls as nonwhite (ie, black, Asian, American Indian, or Hispanic). Black women constituted only 5 of the hemorrhagic stroke cases and only 9 of the controls. Therefore, factor XIII allele frequencies among the black population of Seattle were determined by using a larger number of blood samples obtained from 124 healthy black blood donors from the Puget Sound Blood Center. Compared with the 345 white control subjects from our case-control study, the Leu34 (13% versus 26%, $P<0.001$), Phe204 (0.4% versus 3.0%, $P=0.02$), and Leu564 (15% versus 20%, $P=0.05$) alleles were significantly less common among the 124 healthy blacks. Because of these differences in interracial factor XIII allele frequencies, we excluded the 12 nonwhite hemorrhagic stroke cases and 46 nonwhite control subjects from the present association study to minimize the possibility of confounding due to population admixture. Therefore, our present analysis of the relationship between factor XIII genotypes and risk of hemorrhagic stroke was restricted to the white study subjects with DNA samples (42 hemorrhagic stroke cases and 345 controls).

Results

Characteristics of the Study Subjects

The characteristics of the hemorrhagic stroke cases and controls are summarized in Table 2. The mean age was 37.8 years. The overwhelming majority of the study participants were premenopausal and not using oral contraceptives. Cigarette smoking and hypertension were more commonly reported among hemorrhagic stroke cases than among the

TABLE 2. Characteristics of Hemorrhagic Stroke Patients and Control Subjects

| Characteristic | Hemorrhagic Stroke Cases (n=42) | Control Subjects (n=345) |
|---|---------------------------------|--------------------------|
| Age, y | | |
| Mean | 36.0 | 37.7 |
| Median | 37.0 | 39.0 |
| Range | 18–44 | 19–44 |
| Premenopausal, % | 88.1 | 95.9 |
| Current oral contraceptive use, % | 11.9 | 11.1 |
| Current smokers, % | 47.6 | 20.9 |
| Body mass index >30 kg/m ² , % | 9.5 | 16.3 |
| Hypertension, % | 23.8 | 8.7 |
| Diabetes, % | 4.8 | 3.2 |
| Hypercholesterolemia, % | 16.7 | 16.4 |
| Alcohol consumption ≥ 1 time per week, % | 42.5 | 40.1 |

control subjects. Of the 42 hemorrhagic stroke cases, 30 were classified as SAH, and 12 were classified as ICH. Intracranial aneurysms were documented by imaging studies in 23 (77%) of the 30 women with SAH. Arteriovenous malformations were documented in 4 (33%) of the 12 women with ICH. In the remaining 15 hemorrhagic stroke patients, no vascular source could be identified.

Factor XIII Genotypes, Alleles, and Linkage Disequilibrium

The observed genotype distributions of the factor XIII Val34Leu, Tyr204Phe, and Pro564Leu polymorphisms and the calculated allele frequencies among the white subjects are shown in Table 3. The genotype distributions of all 3 factor XIII polymorphisms were in Hardy-Weinberg equilibrium among the control subjects, indicating the expected relationship between genotype frequencies and calculated frequencies among our study population.

Among the white control subjects, there was a tendency toward negative linkage disequilibrium between the Val34Leu and Tyr204Phe polymorphisms ($D'=-0.58$, $P=0.17$) and between the Val34Leu and Pro564Leu polymorphisms ($D'=-0.19$, $P=0.33$) and a tendency toward positive linkage disequilibrium between the Tyr204Phe and Pro564Leu polymorphisms ($D'=+0.24$, $P=0.16$). Among the 124 black subjects, the negative linkage disequilibrium between Val34Leu and Pro564Leu was considerably stronger ($D'=-0.99$, $P=0.02$).

Association of Factor XIII Polymorphisms With Hemorrhagic Stroke

The genotype distributions and allele frequencies of the Tyr204Phe and Pro564Leu polymorphisms differed significantly among the hemorrhagic stroke cases and the control subjects (Table 3). The age-adjusted OR associated with being a carrier of the Tyr204/Phe204 genotype was 2.9 (95% CI 1.14 to 7.5). There was a 4-fold increased risk of hemorrhagic stroke associated with carrying 2 copies of the Leu564 allele (age-adjusted OR 4.3, 95% CI 1.4 to 13.7) and

TABLE 3. Factor XIII Genotypes in Hemorrhagic Stroke Cases and Controls

| | Alleles | | | Genotypes | | | | OR† (95% CI) | OR† (95% CI) |
|------------------------------------|------------|------------|------|------------|------------|----------|------|-----------------|-----------------|
| | n (%) | n (%) | P* | n (%) | n (%) | n (%) | P* | | |
| Val34Leu | Val | Leu | | Val/Val | Val/Leu | Leu/Leu | | Val/Leu | Leu/Leu |
| Control subjects (n=345) | 512 (74.2) | 178 (25.8) | | 187 (54.2) | 138 (40.0) | 20 (5.8) | | | |
| Hemorrhagic stroke patients (n=42) | 65 (77.4) | 19 (22.6) | 0.53 | 25 (59.5) | 15 (35.7) | 2 (4.8) | 0.42 | 0.8 (0.4–1.5) | 0.7 (0.2–3.2) |
| Tyr204Phe | Tyr | Phe | | Tyr/Tyr | Tyr/Phe | Phe/Phe | | Tyr/Phe | |
| Control subjects (n=345) | 669 (97.0) | 21 (3.0) | | 324 (93.9) | 21 (6.1) | 0 (0) | | | |
| Hemorrhagic stroke patients (n=42) | 77 (91.7) | 7 (8.3) | 0.01 | 35 (83.3) | 7 (16.7) | 0 (0) | 0.03 | 2.9 (1.14–7.5) | . . |
| Pro564Leu | Pro | Leu | | Pro/Pro | Pro/Leu | Leu/Leu | | Pro/Leu | Leu/Leu |
| Control subjects (n=345) | 551 (79.9) | 139 (20.1) | | 218 (63.2) | 115 (33.3) | 12 (3.5) | | | |
| Hemorrhagic stroke patients (n=42) | 57 (67.9) | 27 (32.1) | 0.01 | 20 (47.6) | 17 (40.5) | 5 (11.9) | 0.01 | 1.7 (0.9–3.4) | 4.3 (1.4–13.7) |

*P value for χ^2 test of homogeneity

†OR was adjusted for age. Reference group for OR is women homozygous for the more common allele (ie Val34/Val34, Tyr204/Tyr204 or Pro564/Pro564)

a moderately increased risk associated with carrying 1 copy of the Leu564 allele (age-adjusted OR 1.7, 95% CI 0.9 to 3.4) compared with the Pro564/Pro564 genotype. For subjects who carried at least 1 copy of the Leu564 allele and/or were heterozygous for Tyr204/Phe204 (62% of cases versus 40% of controls), the age-adjusted OR for hemorrhagic stroke was 2.6 (95% CI 1.3 to 5.0). The combined prevalence of the Tyr204/Phe204 genotype and/or the Leu564/Leu564 genotype was 26.2% among the hemorrhagic stroke cases compared 9.3% of the controls (1 case and 1 control carried both genotypes). Thus, women carrying either factor XIII genotype had a 3-fold increased risk of hemorrhagic stroke (age-adjusted OR 3.3, 95% CI 1.5 to 7.1). In contrast, the presence of 1 or 2 copies of the Val34Leu polymorphism was associated with a decreased risk of hemorrhagic stroke (age-adjusted OR \approx 0.7), but the CIs were compatible with the absence of an association (Table 3).

We also assessed whether the associations between each factor XIII polymorphism and hemorrhagic stroke were influenced by the 2 remaining factor XIII polymorphisms with the use of multivariate adjustment. For each factor XIII polymorphism, adjustment for the other 2 did not appreciably affect the univariate risk estimates in Table 3 (data not shown).

For all 3 factor XIII polymorphisms, restricting the analysis to the women who were either premenopausal or were not using oral contraceptives did not appreciably alter the risk estimates for hemorrhagic stroke. When they were analyzed according to hemorrhagic stroke subtype, all 7 hemorrhagic stroke cases carrying the Phe204 allele belonged to the subgroup of 30 women classified with SAH. Thus, the factor XIII Phe204 variant was associated with a nearly 5-fold risk of SAH (age-adjusted OR 4.5, 95% CI 1.7 to 11.8). In contrast, 10 (83%) of the 12 women with ICH carried at least

TABLE 4. Factor XIII Phe204 and Leu564/Leu564 Variants in Hemorrhagic Stroke Cases and Controls, by Other Risk Factors

| Risk Factor | Tyr204Phe Polymorphism | | | | | | Pro564Leu Polymorphism | | | | | |
|------------------------|------------------------|-----|------------------|-----|-----------------|------|------------------------|-----|------------------|-----|-----------------|------|
| | Cases (n=42) | | Controls (n=345) | | OR (95% CI) | P* | Cases (n=42) | | Controls (n=345) | | OR† (95% CI)† | P* |
| | Y/Y | F/Y | Y/Y | F/Y | | | P/P or P/L | L/L | P/P or P/L | L/L | | |
| Overall | 35 | 7 | 324 | 21 | 2.9 (1.14–7.5) | | 37 | 5 | 333 | 12 | 3.5 (1.14–10.6) | |
| Current smoking | | | | | | | | | | | | |
| No | 17 | 5 | 258 | 15 | 5.2 (1.7–16.4) | 0.09 | 22 | 0 | 265 | 8 | 0 (0–6.0) | 0.03 |
| Yes | 18 | 2 | 66 | 6 | 0.9 (0.2–5.5) | | 15 | 5 | 68 | 4 | 5.6 (1.3–23.9) | |
| Hypertension | | | | | | | | | | | | |
| No | 27 | 5 | 297 | 18 | 2.8 (0.95–8.3) | 0.89 | 28 | 4 | 304 | 11 | 3.6 (1.04–12.1) | 0.95 |
| Yes | 8 | 2 | 27 | 3 | 2.5 (0.4–18.9) | | 9 | 1 | 29 | 1 | 4.7 (0.3–87.8) | |
| Alcohol consumption | | | | | | | | | | | | |
| <1 time per week | 18 | 5 | 190 | 13 | 4.1 (1.3–12.8) | 0.43 | 19 | 4 | 195 | 8 | 5.0 (1.4–18.2) | 0.54 |
| \geq 1 time per week | 15 | 2 | 128 | 8 | 1.8 (0.3–9.5) | | 16 | 1 | 133 | 3 | 2.0 (0.2–23.6) | |
| PAI-1 genotype | | | | | | | | | | | | |
| 4G/4G or 4G/5G | 26 | 4 | 253 | 19 | 2.0 (0.6–6.6) | 0.19 | 28 | 2 | 262 | 10 | 1.6 (0.3–7.9) | 0.11 |
| 5G/5G | 9 | 3 | 70 | 2 | 12.8 (1.6–80.0) | | 9 | 3 | 70 | 2 | 11.6 (1.7–79.2) | |

Y indicates tyrosine, F, phenylalanine; P, proline, and L, leucine

*P value for interaction

†OR and 95% CI calculated for Leu564/Leu564 genotype compared with Pro564/Pro564+Pro564/Leu564 genotypes and adjusted for age

1 copy of the Leu564 allele compared with only 127 (37%) of the 345 controls (age-adjusted OR 9.1, 95% CI 1.9 to 42.8).

The risk of hemorrhagic stroke associated with Phe204 and Leu564/Leu564 was modified by current cigarette smoking. The association with the Phe204 variant was largest among nonsmokers, whereas the effect of the Leu564/Leu564 genotype was most pronounced among smokers (Table 4). The risk of hemorrhagic stroke associated with either the Phe204 variant or the Leu564/Leu564 genotype was also particularly high among the subgroup of women with the PAI-1 5G/5G genotype. Among the subgroup of women who carried the PAI-1 5G/5G genotype, the presence of either the Phe204 allele or the Leu564/Leu564 genotype was associated with a nearly 20-fold increased risk of hemorrhagic stroke (age-adjusted OR 18.9, 95% CI 3.8 to 95.1). By contrast, there was no increased risk of hemorrhagic stroke in women with either factor XIII genotype who carried the PAI-1 4G/4G or 4G/5G genotype (age-adjusted OR 1.1, 95% CI 0.6 to 4.6). This resulted in a *P* value for interaction of 0.016.

Discussion

Our findings from a population-based association study among young white women indicate that 2 common genetic variants of coagulation factor XIII, Phe204 and Leu564, are each associated with a 3- to 4-fold increased risk of nonfatal hemorrhagic stroke. Although the 2 intragenic factor XIII polymorphisms were in partial linkage disequilibrium, the associations with stroke were mutually independent. The Leu564 allele has been associated with decreased plasma factor XIII levels,^{10,11} and the Phe204 variant has been associated with reduced specific activity of plasma factor XIII.¹² Decreased clot stability may lead to greater susceptibility to intracranial hemorrhage, particularly among younger individuals, who tend to have lower factor XIII levels.²³

The risk estimates associated with the 2 factor XIII polymorphisms appeared to be modified by cigarette smoking, although in opposite directions. The risk associated with the Phe204 variant was greatest among nonsmokers, whereas the effect of the Leu564/Leu564 genotype was most pronounced among smokers. There was also a suggestion that the risk associated with the Phe204 variant was highest among the women with SAH, whereas the risk associated with the Leu564/Leu564 genotype was most pronounced among the women with ICH. Cigarette smoking has been shown to increase the risk of rupture and hemorrhage in subjects with intracranial aneurysm.^{24,25} However, cigarette smoking is also associated with increased plasma factor XIII levels²³ as well as decreased fibrinolysis.²⁶ Thus, our findings suggest that the detrimental effect on clot or vessel wall stability associated with the Phe204 variant may be "neutralized" in cigarette smokers.

The risk estimate associated with the presence of either the Tyr204/Phe204 or Leu564/Leu564 genotype was particularly large among women carrying the PAI-1 promoter 5G/5G genotype. PAI-1 is a major physiological inhibitor of fibrinolysis, and the 5G/5G genotype has been associated with decreased plasma levels of PAI-1 relative to the 4G/4G genotype.¹⁴ Thus, the reduced clotting activity associated with the Phe204 and Leu564 factor XIII variants may be

compounded by increased fibrinolysis associated with PAI-1 5G/5G, which potentially increases the likelihood of intracranial hemorrhage. In addition, factor XIII and PAI-1 both may participate in vascular remodeling, tissue repair, and angiogenesis.^{7,13,27,28} Delayed wound healing, intracranial hemorrhage, and recurrent miscarriage are common features of inherited factor XIII deficiency,⁶ and an increased prevalence of the factor XIII Phe204 allele was recently noted among women with recurrent miscarriage.¹¹ Thus, genetic variation of factor XIII may affect the occurrence of hemorrhagic stroke through effects on vessel wall stability and aneurysm formation or rupture, as well as decreased clot stability.

ICH and SAH are both more common among blacks than whites, and ICH is particularly prevalent in Asian populations. It is interesting to note that the Leu564 allele (which our data suggest may be more strongly related to ICH) is more common among Japanese than white individuals.²⁹ Because our data indicate that both the Phe204 and Leu564 alleles are less common among blacks than whites, there must be other genetic or environmental determinants responsible for the increased incidence of hemorrhagic stroke among blacks.

The lack of association between the factor XIII Val34Leu polymorphism and risk of hemorrhagic stroke in the present study contrasts with the association reported among elderly British patients with ICH.⁹ However, it should be noted that our patient population was considerably younger and that most of the strokes were due to SAH rather than ICH. Thus, it is possible that the Val34Leu mutation is more strongly related to ICH than SAH. However, another recently published study involving a larger number of subjects with both hemorrhagic stroke subtypes did not find any association between the Val34Leu genotype and the risk of hemorrhagic stroke.³⁰

Several limitations of the present study should be noted. Because hemorrhagic stroke is an uncommon event in women aged <45 years, the number of cases in the present study is small, which may increase the likelihood of a spurious association. The analysis of multiple subgroups also increases the possibility of a false-positive association; therefore, the findings with respect to interaction between factor XIII and other risk factors should be interpreted cautiously. The infrequency of hemorrhagic stroke in young women also precluded the use of a prospective design to test our hypotheses. Thus, we were able to study only women who survived at least 3 months after their strokes. Because hemorrhagic stroke is associated with a high case-fatality rate (26% in the present study), our results may be biased because of the exclusion of fatal cases if a particular factor XIII variant is associated with fatal outcome rather than occurrence of hemorrhagic stroke. Finally, although we limited our analysis to white subjects, we cannot exclude the possibility of a spurious association arising that is due to population admixture.

Another potential limitation of the present study is that citrated plasma samples were not available for measurement of factor XIII clotting activity levels among the study subjects. However, even if such plasma samples had been collected, the retrospective study design might complicate

interpretation of factor XIII levels among the stroke cases measured after the acute event. Because previous studies have demonstrated a relationship between the factor XIII Tyr204Phe and Pro564Leu polymorphisms and plasma factor XIII levels,^{10–12} the determination of factor XIII genotype actually may be preferable to the measurement of factor XIII activity levels in this setting. The advantage of studying genetic variants is that they are “fixed at birth” and, therefore, clearly precede disease onset and are not confounded by fluctuations in plasma activity levels related to the acute cerebrovascular event or by other behavioral and environmental risk factors that influence factor XIII activity levels.²³

In summary, genetic variation of coagulation factor XIII may be important in the occurrence of hemorrhagic stroke in young white women, particularly in combination with other genetic and environmental risk factors. These findings require confirmation in larger studies, which should include fatal and nonfatal hemorrhagic stroke cases, other ethnic groups, and family-based studies that assess genetic linkage as well as association.

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Editorial Comment

Hematology-Neurology Connection: Association Between Factor XIII and Hemorrhagic Stroke in Young Women Through Genetic Polymorphism

Coagulation factor XIII (FXIII) belongs to the enzyme family of transglutaminases that catalyze the formation of covalent ϵ -(γ -glutamyl)lysine (amide) bonds on cross-linked proteins.¹ Protein cross-linking is involved in several physiological processes, including hemostasis, wound healing, and tumor growth. FXIII is a proenzyme that is activated by thrombin during the late stage of blood coagulation. It is a heterotetramer consisting of 2 catalytic A (FXIIIA) and 2 noncatalytic B (FXIIIB) subunits. On thrombin activation, the enzyme (ie, activated FXIII) catalyzes the formation of covalent bonds among fibrin monomers. Activated FXIII also promotes clot retraction² by binding to platelet GPIIb/IIIa receptors (attached to platelet microkeleton via its cytoplasmic domain) and cross-links fibrin with a number of other proteins, including fibronectin and α_2 -antiplasmin. The end result is the formation of sturdier thrombi, more resistant to shear- and fibrinolysis-induced lysis. These actions of activated FXIII explain in part why older thrombi are more difficult to lyse with thrombolytic agents. FXIII has a long plasma half-life of 6 to 10 days, and limited amounts are required for normal hemostasis (0.05 U/mL). Congenital FXIII deficiency is exceedingly rare. It is inherited as an autosomal recessive trait and caused by absence of either subunit. Deficiency of the FXIIIA subunit is the most common, however. Homozygotes have a lifelong bleeding diathesis, typically starting with bleeding from the umbilical cord at birth and later characterized by delayed bleeding after trauma or surgery and delayed wound bleeding. During childhood, patients bruise easily and experience subcutaneous and intramuscular hematomas. Spontaneous abortion has been reported in affected females, presumably owing to defective formation of a cytotrophoblastic shell.³ Hemorrhage into the central nervous system occurs more frequently than with other inherited coagulation defects. Other conditions involving defective fibrin formation (eg, disseminated intravascular coagulation) or fibrin breakdown (eg, thrombolytic administration) are also associated with central nervous system bleeding. These observations suggest the theme that faulty fibrin formation or fibrin breakdown is associated with central nervous system bleeding. Most cases of congenital FXIII deficiency are due to mutations in the subunit A gene, often involving point mutations causing amino acid substitution. In addition, several single nucleotide polymorphisms of the FXIIIA gene resulting in single amino acid substitutions have been described. Genetic polymorphisms are typically encountered in at least 1% of the normal population. The dissection of human disease through genetic polymorphisms has become an important research area of vascular biology and other disciplines in recent years.

In the preceding article, Reiner et al examined the association between the Val34Leu, Tyr204Phe, and Pro564Leu variants of FXIIIA and the risk of nonfatal hemorrhagic stroke in young women by conducting a population-based case-control study. They also examined possible gene-gene interactions between FXIIIA polymorphisms and the 4G/5G promoter polymorphism of the PAI-1 gene. PAI-1 is the main inhibitor of tissue plasminogen activator and is released from activated endothelial cells and the α -granules of activated platelets. The main results show that women carrying the Tyr204/Phe204 or Leu564/Leu564 genotype have a significantly higher risk of hemorrhagic stroke compared with control subjects (age-adjusted ORs of 2.9 and 4.3, respectively). This risk is further magnified when either genotype is combined with the PAI-1 5G/5G alleles (age-adjusted OR 18.9). The authors conclude that the Phe204 allele and Leu564/Leu564 genotypes may be markers of genetic susceptibility to nonfatal hemorrhagic stroke in young white women.

A number of aspects emerging from this well-designed study deserve mentioning. First, the investigators selected the Tyr204Phe and Pro564Leu genotypes their phenotype associated with decreased plasma FXIII levels. This design feature underscores the fundamental principle that for a gene change to have an impact, it must be mediated through a phenotype in turn associated with a clinical effect. Studies of association between polymorphisms and disease without reference to phenotype may lead to false associations, influenced by other extraneous factors. Second, in contrast to similar studies in older patients, the Leu34 allele is not associated with an increased hemorrhagic risk. The authors point out the Leu34 allele may be more closely related to the risk of intracerebral bleeding than of subarachnoid hemorrhage, the most common type encountered in their study of younger women. Other case-control studies^{4,5} in ischemic brain syndromes and in myocardial infarction have yielded negative associations between FXIIIA-Leu34 and these disease outcomes. Recent *in vitro* studies⁶ have shown that FXIII containing Leu34 is activated more rapidly by thrombin and results in altered fibrin with reduced tensile strength. Thirdly, the gene-gene interaction observed between Tyr204/Phe204 or Leu564/Leu564 and PAI-1 5G/5G genotype (but not 4G/5G) is biologically plausible. The PAI-1 4G/5G genotype has been associated in some studies with increased PAI-1 activity in plasma (causing reduced fibrinolysis) and increased risk of myocardial infarction or ischemic stroke. Conversely, the PAI-1 5G/5G genotype has been associated with reduced PAI-1 activity, possibly causing increased fibrinolytic activity. Collectively, the current report and other

studies raise the hypothesis that some FXIIIa polymorphisms may be somewhat protective against thrombosis but are perhaps involved in the pathogenesis of hemorrhagic disorders. These preliminary findings will need to be investigated further in future studies.

In view of their widespread availability, molecular biology techniques have now entered the clinical trial arena. Studies of genetic polymorphisms abound in practically all disciplines of clinical research. The underlying principle is based on the notion that phenotypic variations among individuals, including anthropometrics, disease susceptibility, and response to the environment, are mostly due to naturally occurring variations in DNA sequence, consisting of single nucleotide polymorphisms (SNPs). SNPs are evenly distributed throughout the genome and thus likely flank or are nearby coding genes.⁷ Association studies examine whether polymorphic alleles occur more frequently in cases than in controls. If a significant association occurs, either the polymorphism is closely associated with the susceptibility locus or is in linkage disequilibrium. However, these associations do not necessarily imply a causality link with the disease under study and, like any other case-control study, are susceptible to bias. The genetic markers identified through studies must be viewed as risk factors, albeit at the molecular level. Two advantages of genetic markers over epidemiological risk factors or biochemical markers are that their exposure always precedes the disease of interest and the underlying disease cannot influence test results. These 2 factors are common sources of bias in classic case-control studies. The

validity of polymorphisms as genetic markers can be ascertained only through several studies showing consistent results. Perhaps one of the best examples to cite was the demonstration of the angiotensin-converting enzyme insertion/deletion (I/D) polymorphism as marker of susceptibility to cardiovascular disease.

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